U.S. EPA National Risk Management Research Laboratory
Treatment and Destruction Branch

Innovative Measures of Treatment Performance

Benefits of Using Innovative Measures

Several monitoring techniques and methods have been adapted and are used to evaluate the performance of remedial technologies and processes treating soils and sediments. Traditionally, treatment effectiveness was determined by changes in contaminant concentration. While contaminant concentration continues to be an important measure for our research, additional assays provide further insight into the remedial treatments. For example, many bioremediation treatments rely on soil microbes to degrade contaminants. Phospholipid Fatty Acid Methyl Ester Analysis (PL-FAME) can provide information about the composition, size, and physiological status of the microbial community. Isotope Ratio Mass Spectrometry (IRMS) can be used to differentiate the carbon sources supporting microbial metabolism. Oxygen Uptake Rate measurements assist in understanding the metabolic activity of the microbial community. Bioassays using soil organisms provide information about whether remediated materials are compatible with plant germination and growth, or in other words, is the remediated soil environmentally viable. Using these measurements and others, a fuller understanding of the treatment processes and treatment effectiveness is gained. A summary of innovative measures is presented in Table 1 including sample size required, matrices tested in our branch, and relative difficulty.

Table 1. Assays Used to Evaluate Treatment Performance

Assay	Sample Size	Matrix	Difficulty (1-5)
PL-FAME	>2 g or 5 mL	soil, sediment, slurry or water	4
TC-FAME	5 g for culture	soil, sediment	2
16S rRNA		soil, sediment	5
IRMS	10 g	soil, sediment, water, gas	4
OUR	60 g	soil, sediment	1
Solid Phase Microtox	20 g	soil, sediment	2
Earthworm survival	3 kg	soil, sediment	3
Seed Germination and Root Elongation	3 kg	soil, sediment	3
PBEP	20 g	soil, sediment	4

Microbial Community Characterization

Many remediation technologies depend on the activity of the microbial community to either remove or degrade a contaminant of interest. Historically, little attention has been paid to understanding how the microbial community responsible for the degradation reacted to treatment conditions and how their response changed over time. Decisions about treatment effectiveness were generally based on gross measures of contaminant removal. Assays such as PL-FAME enhance our understanding of the composition and dynamics of the microbial community. Other community assays compliment PL-FAME and help in developing a more complete picture of the structure, type, number, abundance, and physiological status (i.e., stress, age, viability) of the microbial community. Armed with this knowledge, researchers can evaluate treatment effects, explain differing results, compare treated soils to clean ones, and develop directed treatments to maintain and enhance the removal activity of the microbial community.

Phospholipid Fatty Acid Methyl Ester Analysis (PL-FAME) - PL-FAME is a biochemical technique that provides direct information about the general structure and activity of a microbial community, without the limitations associated with culturing microorganisms. In this assay, an environmental sample is extracted, and a microbial community fingerprint is established based on the phospholipid fatty acid composition. This fingerprint represents the living portion of the microbial community since phospholipids are rapidly degraded following cell death. The

ability to generate a fingerprint helps in determining changes that take place within a microbial community during treatment and between treatments. Using the PL-FAME technique, certain signature fatty acids can be used to establish a relative physiological status of the community (i.e., stress, age) and infer the general make-up and changes of gross groupings of microorganisms (eukaryote, Gram+, Gram-,and fungi) in the microbial community. (Contact Ronald F. Herrmann, 513/569-7741, herrmann.ronald @epamail.epa.gov)

Total Cellular Fatty Acid Methyl Ester Analysis (TC-FAME) - In classic microbiology, microbes were identified by isolating pure cultures and characterizing metabolic capabilities and phenotypic characteristics. However, these classical techniques are of limited value for environmental samples since less than 1% of environmental organisms are culturable through classical techniques. As a result, classical techniques would yield a distorted picture of the microbial assemblage. TC-FAME can be used to identify microbial characteristics while retaining qualitative information about the community structure. TC-FAME is similar to PL-FAME in that cellular lipids are used to identify microbes. However, instead of focusing on phospholipids, TC-FAME considers the total cellular lipids. These lipids are extracted for microbial isolates, saponified, and methylated. Gas chromatography is used to identify the fatty acid composition of unknown isolates. By comparing the fatty acid profiles from unknown samples to libraries containing the fatty acid profiles from many organisms, the unknown members of the microbial community can be identified. In some cases, pure cultures may be studied. (Contact Kim McClellan, 513/569-7214, mcclellan.kim@epamail.epa.gov)

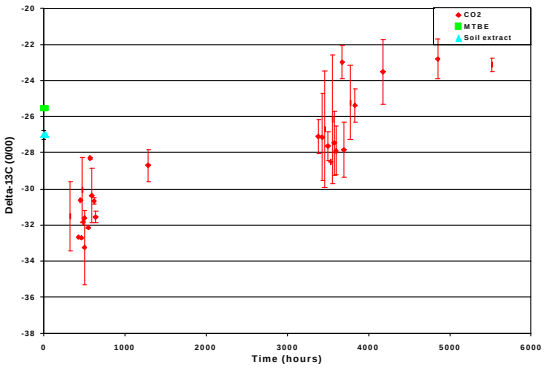
16S rRNA Community Structure Analysis (16S rRNA) - In recent years, significant progress has been made in the field of molecular biology enabling the identification of microbes in complex environmental samples without the need to culture these organisms from the sample. Specifically, the DNA sequence of a gene coding for the 16S ribosomal RNA sub-unit (16S rRNA) can be determined and compared to known sequences to identify microbial species present in the sample. In our lab, we are extracting genomic DNA from soil or sediment samples from sites impacted by oil pollution. The region of the genome of particular interest, the 16S rRNA sequence, is copied and amplified from the mixture of microbial genomic DNA using the polymerase chain reaction (PCR) and primers specific to the region of interest only. The resulting mixture of 16S rRNA fragments is then cloned to isolate and amplify fragments from a proportion of the individual species. Sequence data is generated and then compared to databases of several thousand known 16S rRNA sequences to identify the unknown microbes in the sample. There are limitations to this approach. The rich diversity of microbes present in a typical soil or sediment sample dictates that a large number of sequences must be determined in order to produce an accurate picture of the sample's microbial ecosystem. In addition, the extraction and sample handling procedures are extensive. (Contact Ronald F. Herrmann, 513/569-7741, herrmann.ronald @epamail.epa.gov)

Microbial Metabolism Characterization

In addition to understanding the composition of the microbial community, tools which characterize microbial metabolism are also useful. For example, IRMS can distinguish contaminant degradation from other metabolic activity. Using this information, researchers can better understand the effects of various treatments on contaminant degradation and other microbial reactions. This information may also assist in identifying problems and develop treatments which target specific biochemical activities.

Isotope Ratio Mass Spectrometry (IRMS) - IRMS is a technique which measures the ratios of stable isotopes from a sample. For example, the ratio of naturally occurring C^{12} and C^{13} can be determined for an agricultural soil sample. This information provides insight into the types of biological reactions relevant to the soil such as which type of photosynthesis predominated. In addition, IRMS can be used to understand microbial metabolism. In some cases, the degradation of a petrochemical in the environment can give rise to metabolic products such as CO_2 that have an isotopic ratio different than that found from degradation of native plant material deposited in the environment. If the differences are great enough, the proportion of CO_2 coming from the pollutant relative to the native organic matter can be assigned. The resulting information can be used to generate kinetic data as well as proof of complete degradation of the pollutant.

IRMS methods can be used to analyze gas samples, semivolatile materials, and nonvolatile samples. The sample size and handling requirements for this type of analysis vary widely depending on the data needed and the sample matrix. In sample handling, one must control the sample to prevent isotope discrimination in the sample processing. Gases are most vulnerable. Slight temperature variations can introduce wide variations in results. Homogenization of the sample matrix is vital for good results. In addition, extensive quality control samples are required. Working standards must be selected with an isotope ratio close to the analytes being examined. Results are usually required to have a standard deviation in delta value of less than 0.1 parts per thousand. Larger variations are unacceptable. (Contact John R. Haines, 513/569-7446, haines.john @epamail.epa.gov)



δ-^{13}C signatures of CO_2 versus time in aerobic respirometers

Oxygen Uptake Rate Measurements (OUR)- When microbes aerobically metabolize carbon and energy sources, oxygen is consumed proportionally. Using the OUR technique, small quantities of contaminated soil are placed in sealed flasks and O_2 and CO_2 headspace concentrations are monitored for about 1 week. The rate of O_2 uptake and CO_2 evolution indicates the metabolic activity of the soil. By tracking OUR over the course of a treatment, researchers can observe changes in general microbial activity. This information helps researchers monitor the availability of microbial carbon sources (such as contaminants) and understand if adverse conditions such as a pH drop are inhibiting microbes. While OUR cannot distinguish between degradation of contaminants and natural organic matter, the way IRMS can, OUR is a relatively simple and robust technique. (Contact Gregory Sayles, 513/569-7607, sayles.gregory@epamail.epa.gov)

Bioassay Assessment of Treatment Effectiveness

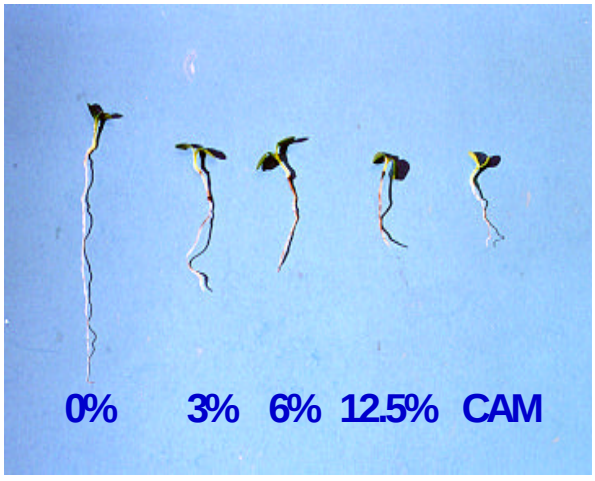


Figure 2. Typical dose-response curve of lettuce root elongation as a function of the fraction of Reilly Tar soil added to a clean dilutant soil. CAM is a positive control.

In our branch, bioassay testing is typically conducted before and after remedial treatment in conjunction with chemical measurements. In this way, researchers can understand if treatments reduce toxicity as well as chemical concentrations. Bioassay testing can strengthen the evaluation of remedial treatment performance in several ways. First, since bioassay testing evaluates the aggregate effect of the sample on reporting organisms, aspects such as soil matrix effects, sorption/desorption behavior, and chemical mixture interactions are included in the evaluation of treatment effectiveness. Thus, bioassay testing can be a more reliable way of describing the potential hazard than estimates based on chemical concentration alone. Secondly, bioassay testing is not biased by assumptions about the primary sources of toxicity, and results can be compared to chemical based assessments to evaluate whether sources of toxicity have been omitted from characterizations. Finally, by evaluating samples before and after treatment, increased responses due to incomplete treatment or toxicity introduced with process amendments can be identified. For example, in many bioremediation processes, incomplete degradation of target contaminants is a common concern. Toxicity, measured before and after

treatment, provides a rational basis for evaluating whether treatment is effective. It would be difficult to monitor bioprocesses in any other way due to the large number of degradation products that can be formed.

For soil and soil-like materials, bioassay testing has focused on four assays:

- 14-day earthworm survival with *Eisenia fetida*;
- 5-day seed germination and root elongation with *Lactuca sativa* and *Avena sativa*; and
- the solid phase Microtox assay.

The earthworm and plant assays were selected because they are: relevant to soil toxcity; include plant and animal reporter species; have acute and chronic exposure periods; and evaluate mortality and growth endpoints. In addition, these assays are practical for the facilities available and processes used. The Microtox assay has been included since it is relatively easy and quick to use and uses a small samples size. In fact, due to the samples size and ease of use, the Microtox assay has been used to test samples throughout the course of treatment. In a few cases, additional assays have been used to evaluate treatments. Colleagues from other laboratories in EPA ORD have contributed to these studies. The following assays have been used:

- Ames assay (Kathleen Schenck and Thomas Hughes);
- Allium Mitotic Aberration Assay (John Meier);
- *Hyalella azteca* 7 or 10 -day mortality and growth tests,
  Lumbriculus variegatus 7 -day mortality and budding tests,
  Pimephales promelas embryo- larval 8 -day mortality and teratogenic tests,
  Lemna minor 4-day frond number, growth as wet weight and Chlorophyll-a tests, and
  Ampelisca abdita 10 -day Mortality tests (James Lazorchak)

(Lina Chang conducted initial earthworm and plants tests). (Contact Carolyn Acheson, 513/569-7190, Carolyn.Acheson@epamail.epa.gov)

Characterizing Contaminant Desorption under Physiological Conditions

Physiologically Based Extraction Procedure (PBEP) - Traditionally, remedial technology performance has been evaluated based on the total amount of extractable contaminants. Since methods which measure extractable soil contaminants bear little resemblance to human digestive processes, this evaluation may overestimate or underestimate the actual accessability of environmental contaminants to humans via ingestion. Rather than estimate human exposure either by general approximations or animal models, contaminant desorption from soil could be measured using an extraction process which simulates the function of the human gastrointestinal tract. Such an extraction, the PBEP, is being developed. The current approach involves sequential simulation of the various organs of the GI tract using liquids and conditions which mimic physiological conditions. This research is focusing on the desorption of organic contaminants such as polycyclic aromatic hydrocarbons. (Contact Carolyn Acheson, 513/569-7190, Carolyn.Acheson@epamail.epa.gov)